REMARKS/ARGUMENTS

I. Status of the claims

Claims 1-11 and 15 are pending. No new matter is added.

II. Interview

Applicants appreciate the opportunity to discuss the case with Examiner Jagoe on January 7, 2009. The focus of the interview was the obviousness rejection based on Schatzberg, US Patent No. 6,150,349 (the '349 patent). Applicant's representative Carol Johns argued that the '349 patent teaches that psychotic symptoms resulting from glucocorticoid dysregulation can be treated with a glucocorticoid receptor antagonist (GRA), but that not all disorders with psychotic symptoms result from glucocorticoid dysregulation. The '349 patent discloses two examples of psychotic conditions that do not result from glucocorticoid dysregulation, schizophrenia and manic disorders, and states that these conditions are therefore not treatable with a GRA. Applicant's representative stated that one of skill would not have a reasonable expectation, given this disclosure, that a GRA would successfully treat any type of psychosis.

Examiner Jagoe pointed to the disclosure of postpartum psychosis on column 15 of the '349 patent, and stated that this disclosure indicates that the condition is treatable with a GRA. Ms. Johns explained that this section, entitled "Diagnosing and assessing conditions and illnesses involving psychosis," provides a general list of psychotic conditions that is not limited to those resulting from glucocorticoid regulatory dysfunction.

Examiner Jagoe stated that, unless the reference *specifically excludes* the condition, it would be obvious to try to treat the psychotic symptoms with a GRA. This standard is repeated in the Interview Summary issued January 12, 2009.

No agreement was reached during the interview.

III. Rejection under 35 USC § 112, first paragraph – Written description

The Examiner has rejected claims 3 and 4 as allegedly lacking written description. According to the Examiner, the terms "steroidal skeleton," "phenyl-containing moiety," and

"dimethylaminophenyl moiety" are unclear because there are no examples or drawings of these structures in the specification. The Examiner cites a definition for moiety that is allegedly from IUPAC as follows: "a half of a molecule including substructures of functional groups." Applicants respectfully traverse the rejection for the reasons set forth below.

As set forth in § 2163 of the MPEP, written description requires that the specification describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention. As indicated by the Examiner, "an applicant shows possession of the claimed invention by describing the claimed invention with all of its limitations using such descriptive means as ***words***, structures, figures, diagrams, and formulas that fully set forth the claimed invention." MPEP § 2163, Part I, *citation omitted.* The Examiner bears the burden of establishing a *prima facie* case by explaining why a person skilled in the art at the time the application was filed would not have recognized that the inventor was in possession of the invention as claimed in view of the disclosure of the application as filed.

As an initial matter, Applicants submit the following description of "moiety" obtained from the IUPAC website: "a part of a molecule." A printout of the description is provided as **Ex. A**, and the website address is as follows: http://old.iupac.org/publications/compendium/index.html. Applicants are unable to find the definition cited by the Examiner that indicates that a moiety is half of a molecule.

The structures of steroid compounds, including cortisol, were well-known at the time of filing. In fact, the naming convention for steroids was established at least as early as 1971. **Ex. B** is a excerpt from "Definitive Rules for Nomenclature of Steroids," published by IUPAC in 1971. Pages 287-288 describe numbering of the carbons in the backbone, and α and β orientation.

The specification, starting at page 9, line 21, describes steroidal glucocorticoid receptor antagonists (GRA), including various modifications that can be made to the cortisol steroid structure to generate a GRA. These structures and modifications are described using the standardized IUPAC naming convention that was familiar in the chemical art at the time of filing, and acknowledged by the Examiner. Thus, these words would be understood by one of

skill to describe chemical structures. The section also cites references that describe examples of these modified steroid compounds.

The Examiner has therefore failed to explain why one of skill would not immediately envision a steroidal compound with an 11β phenyl moiety, given the familiar IUPAC nomenclature. One of skill, reading the present disclosure and claims 3 and 4, would understand the description of the recited compounds and recognize that the inventors were in possession thereof.

The Examiner also alleges that the specification does not provide examples of GRAs with a steroid skeleton and at least one phenyl-containing moiety or at least one dimethylaminophyenyl moiety in the 11β position. Yet mifepristone is just such an example. The structure of mifepristone was well-established at the time of filing. The systematic IUPAC name for mifepristone is ***11β-[p-(Dimethylamino) phenyl]***-17β-hydroxy-17-(1-propynyl)estra-4,9-dien-3-one (*see* page 10, lines 25-26 of the specification).

The chemical structures recited in claims 3 and 4 are described following a familiar, standardized naming convention. There is no evidence that one of skill in the chemical arts would not reasonably believe that the inventors were in possession of the described compounds. Accordingly, Applicants respectfully request withdrawal of the rejection under the first paragraph of 35 USC § 112 for written description.

IV. Rejection under 35 USC § 103 – US Patent No. 6,150,349 (the '349 patent)

The Examiner has rejected claims 1-6 and 9-11 as allegedly obvious over the '349 patent. According to the Examiner, the '349 patent teaches use of GRAs for treatment of psychosis, including psychotic disorders not otherwise specified. The Examiner includes postpartum psychosis in this category, and concludes that it would have been obvious to treat the symptoms of postpartum psychosis with GRAs. The Examiner makes what appears to be the same rejection based on passages from US Patent No. 6,362,173 (the '173 patent) that are identical to those from the '349 patent. Applicants therefore treat the rejections based on the '349 patent and '173 patent as equivalent and direct the following arguments to both rejections.

The substance of the January 7, 2009 interview is summarized above. Applicants noted that, while postpartum psychosis (PPP) is disclosed in the '349 patent, it is disclosed in a section that describes psychotic conditions generally, *i.e.*, not limited to those conditions treatable with a GRA. The Examiner responded that, because the '349 patent does not *specifically exclude* PPP, it would be obvious to treat the psychotic symptoms of PPP with a GRA.

Applicants respectfully traverse the rejection as based on an **incorrect standard** for obviousness. The Examiner has failed to make a proper *prima facie* case of obviousness because there is no explanation of why one of skill in psychological disorders would reasonably expect a GRA to successfully treat the particular psychotic symptoms related to the postpartum condition. The Examiner has not shown that the art was aware of a medically-accepted link between glucocorticoid regulatory dysfunction and PPP.

The '349 patent, while providing a broad definition of psychosis and a general list of conditions with psychotic symptoms, clearly limits the scope of the invention to psychotic symptoms caused by glucocorticoid regulatory dysfunction. The '349 patent does not suggest that glucocorticoid signaling is related to the psychotic symptoms that occur in rare cases after a woman gives birth. While the art appreciated that psychotic symptoms result from a number of different etiologies, the etiology of PPP was not known at the time of filing. Moreover, the art teaches away from treating a new mother, with rapidly declining cortisol levels, with an inhibitor of glucocorticoid signaling, *i.e.*, a GRA.

The present claims are not obvious under the proper standard because one of skill would have no reason to expect that the psychotic symptoms of PPP would respond to GRAs. This argument is explained in more detail below.

A. A prima facie case of obviousness requires articulation of the reasons for a reasonable expectation of success

As explained in the MPEP 2143.02, a *prima facie* case of obviousness requires reasonable predictability or a reasonable expectation of success in practicing the claimed

invention (*see also* examples in MPEP 2143). Whether an art is predictable or provides a reasonable expectation of success is determined at the time the invention was made.

The MPEP 2142 explains that the examiner must step backward in time and into the shoes worn by the hypothetical person of ordinary skill in the art when the invention was unknown. The burden is on the examiner, in view of all factual information, to provide some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness. Rejections for obviousness cannot be sustained with mere conclusory statements.

B. The Examiner has not applied the proper standard for obviousness to the claims

Applicants find nothing in the case law or MPEP stating that non-obviousness is found only where the prior art teaches away from a claimed invention. As explained above, the issue of obviousness turns on predictability, as determined from the point of view of one of skill in the art. The burden is on the examiner to explain why the claimed invention would have been predictable at the time of the invention.

Using the obviousness standard articulated by the Examiner, an examiner would merely have to assert that the art does not exclude the possibility of the invention. The burden is therefore placed on the **applicant** to demonstrate non-obviousness, presumably with rebuttal evidence teaching away from the claimed invention. This is not the law. As explained in more detail below, however, the art does teach away from treating a new mother with a GRA.

C. The Examiner has not shown that one of skill would reasonably expect a GRA to successfully treat the psychotic symptoms of postpartum psychosis

As explained above, a *prima facie* case of obviousness requires demonstration that one of skill in the art would have a reasonable expectation of success in practicing the claimed invention. The references and common knowledge in the art must logically lead in some way to the claimed invention.

The presently claimed methods are not obvious under the proper standard because there is no evidence one of skill would reasonably expect a GRA to successfully treat the psychotic symptoms of PPP.

Until the Examiner provides evidence from the relevant time period to support a medically-accepted link between glucocorticoid regulatory dysfunction and PPP, there can be no *prima facie* case of obviousness based on the '349 patent. As discussed below, the etiology of PPP was unknown at the time of filing.

We have explained several times during the prosecution of this application that the '349 patent is directed to "a method for treating psychosis whose pathogenesis is related to glucocorticoid regulatory dysfunction" (*see* column 1, lines 24-26). Yet the '349 patent defines "psychotic" in its "broadest sense" (*see* column 5, line 37-38).

The '349 patent explains that not all psychotic symptoms are a result of glucocorticoid regulatory dysfunction. Column 6, lines 63-66 states:

> In contrast, patients with, ***e.g.***, schizophrenia... and manic states do not have glucocorticoid regulatory dysfunction.
>
> *Emphasis added.*

Schizophrenia and manic states are *examples* of conditions that are not related to glucocorticoid regulatory dysfunction. The disclosure continues in column 7, lines 10- 11, to explain that "schizophrenia and manic states are not treatable by the methods of the invention."

As part of defining psychosis in its broadest sense, the '349 patent includes a general section on "Diagnosing and assessing conditions and illnesses involving psychosis," where many of the psychotic conditions from the DSM-IV (cited in the '349 patent) are disclosed, including PPP.

However, nothing in the '349 patent links glucocorticoid regulatory dysfunction to the psychotic symptoms of PPP. The '349 patent does not single out PPP for GRA treatment from the broadly defined list of psychotic conditions. Instead, the '349 patent discloses that not all psychotic conditions are related to glucocorticoid regulatory dysregulation.

D. The underlying etiology for psychotic symptoms of postpartum psychosis was unknown

As explained in the Response filed February 13, 2008, there is no agreement in the medical community concerning the physiological basis for psychotic symptoms during the postpartum period.

Applicants include as **Ex. C** excerpts from the DSM-IV, published by the American Psychiatric Association in 2000, relating to "Schizophrenia and other psychotic disorders." The second paragraph of this section (page 297) explains that the term "psychotic" has received a number of different definitions, none of which have been universally adopted. The manual groups a number of disorders with psychotic symptomology together, despite the fact that they display psychotic symptoms to a different extent and do not have a common etiology.

Page 343 includes a brief summary of "Psychotic Disorder Not Otherwise Specified." This section, which includes PPP, lists psychotic symptomologies about which there is "inadequate" or "contradictory" information.

This disclosure indicates that those of skill in psychological disorders did not know the physiological basis for PPP. Those of skill were, however, aware that psychotic symptoms have different causes and cannot all be treated in the same way.

A reasonable expectation of success is determined based on the hypothetical person of skill at the time, without benefit of hindsight. The question here is what one of skill in psychological disorders would consider reasonable when faced with a woman experiencing PPP. Our past responses have noted the severity of this rare disorder – affected new mothers can lose touch with reality (*see, e.g.*, February 13, 2008 response and attached Exhibits).

The Examiner has not articulated why one of skill, with no link between glucocorticoid signaling and PPP, and knowing that psychotic symptoms can arise from any number of different causes, would be reasonably motivated to apply a drug that is limited to one category of psychotic conditions to the particular and rare psychotic symptoms of PPP.

E. The art teaches away from treating a postpartum mother with an inhibitor of glucocorticoid signaling

The prior art teaches that cortisol levels fall dramatically immediately after birth. The claimed method involves inhibition of cortisol signaling, *i.e.*, using a glucocorticoid receptor antagonist. One of skill would not be motivated to treat a woman immediately postpartum with an antagonist of a hormone that is already in rapid decline.

Ex. D (Elendov *et al.* (2001) *J. Clin. Endocrinol. Metab.* 86:4933-38). The last paragraph on the first page explains that cortisol, as well as other hormones, increases in late pregnancy and falls rapidly in the early postpartum period (page 4933, last paragraph). **Ex. E** (Hendrick *et al.* (1998) *Psychosomatics* 39:93-101) dedicates an entire section to cortisol in the postpartum period. **Ex. E** confirms that cortisol levels fall abruptly at delivery (page 97, col. 2).

The relevant art indicates that cortisol levels fall dramatically after birth. Thus, absent some evidence that glucocorticoid regulatory dysfunction was linked with the psychotic symptoms of PPP, one of skill would not be motivated to treat a postpartum mother with an inhibitor of cortisol signaling. As explained above, the Examiner has yet to provide this evidence, as required for a *prima facie* case of obviousness.

F. Conclusion

The '349 patent limits the use of GRAs to treatment of psychotic conditions that result from glucocorticoid regulatory dysfunction. The art taught that psychotic symptoms result from a number of different etiologies. However, the physiological basis for PPP was unknown at the time, and there is no evidence that one of skill would have linked PPP to glucocorticoid regulatory dysfunction. In fact, the art taught that cortisol levels decline rapidly after birth. One of skill would therefore not be motivated to treat a new mother with a GRA.

The Examiner has not articulated why one of skill would reasonably link the psychotic symptoms of PPP with glucocorticoid signaling, as is required to establish a *prima facie* case of obviousness. In view of the foregoing, Applicants respectfully request withdrawal of the rejections under 35 USC § 103 over the '349 patent and the '173 patent.

V. Rejection under 35 USC § 103 – the '349 patent (and '173 patent) and Belanoff

The Examiner has rejected claim 15 as allegedly obvious over the '349 patent in view of Belanoff. According to the Examiner, Belanoff supplies the information that mifepristone is a specific GRA. Again, the Examiner makes what appears to be the same rejection based on the '173 patent in view of Belanoff. Applicants do not see any difference between these rejections, thus, both rejections are addressed here.

Applicants rely on the arguments set forth above for independent claim 1, namely that the '349 patent does not establish a proper *prima facie* case of obviousness. The addition of the Belanoff reference does not lend further support to the Examiner's position.

For the reasons set forth above, Applicants respectfully request withdrawal of the rejections under 35 USC § 103 over the '349 patent in view of Belanoff and the '173 patent in view of Belanoff.

VI. Rejection under 35 USC § 103 – the '349 patent, Stowe, and Bradley

The Examiner has rejected claim 7 based on the '349 patent in view of Stowe and Bradley (*i.e.*, Morgan *et al.*). According to the Examiner, the '349 patent and Stowe do not teach the particular GRA recited in the claim. There is no explanation of what Stowe, a review about postpartum depression, does teach or why it is cited. The Examiner asserts that Bradley teaches the recited GRA.

As explained above, the primary reference does not provide a reasonable expectation that a GRA would successfully treat the psychotic symptoms of PPP. Nothing in Stowe links glucocorticoid regulatory dysfunction to PPP. As explained in detail in the February 13, 2008 response and associated exhibits, Stowe focuses on postpartum depression, which is widely recognized as a distinct disorder from PPP. Bradley also fails to link glucocorticoid regulatory dysfunction to PPP.

If claim 1 is non-obvious over the '349 patent, all the dependent claims must be non-obvious. Applicants therefore respectfully request withdrawal of the rejection of claim 7 under 35 USC § 103 based on the '349 patent, Stowe, and Bradley.

VII. Rejection under 35 USC § 103 – the '349 patent, Stowe, and Gebhard

The Examiner has rejected claim 8 based on the '349 patent in view of Stowe and Gebhard. The Examiner states that the '349 patent and Stowe do not teach the particular GRA recited in the claim, but again does not explain why Stowe is cited. The Examiner asserts that Gebhard teaches the GRA recited in claim 8.

Again, Applicants rely on the arguments above to submit that the Examiner has not established a *prima facie* case of obviousness with the '349 patent. If claim 1 is non-obvious over the '349 patent, all the dependent claims must be non-obvious. In view of the foregoing, Applicants respectfully request withdrawal of the rejection of claim 8 under 35 USC § 103 based on the '349 patent, Stowe, and Gebhard.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

Carol P. Johns
Reg. No. 50,463

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, Eighth Floor
San Francisco, California 94111-3834
Tel: 415-576-0200
Fax: 415-576-0300
Attachments: Ex. A-E
CPJ:cpj
61740359 v1

I U P A C

IUPAC Compendium of Chemical Terminology

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Ex. A

[A] [B] [C] [D] [E] [F] [G] [H] [I] [J] [K] [L] [M]

[N] [O] [P] [Q] [R] [S] [T] [U] [V] [W] [X] [Y] [Z]

The pdf online version of the IUPAC Compendium of Chemical Terminology mostly corresponds to the second edition (1997), compiled by Alan D. McNaught and Andrew Wilkinson (Royal Society of Chemistry, Cambridge, UK). Towards the end of 2003, work began on the addition of terms from more recent IUPAC recommendations, with the intention of eventually bringing the website into a condition in which it can be maintained up-to-date.

Some minor errors have been corrected (the changes are noted where they occur), and cross-referencing has been improved. The conversion to electronic form (pdf files) was carried out by David Stout (Information Technology Consultant, Information Services, Royal Society of Chemistry).

moiety

In physical organic chemistry moiety is generally used to signify part of a molecule, e.g. in an ester R^1COOR^2 the alcohol moiety is R^2O. The term should not be used for a small fragment of a molecule.

1994, *66,* 1141

INTERNATIONAL UNION OF
PURE AND APPLIED CHEMISTRY
AND
INTERNATIONAL UNION OF BIOCHEMISTRY

DEFINITIVE RULES FOR NOMENCLATURE OF STEROIDS

EXB

Issued by the
IUPAC Commission on the Nomenclature of Organic Chemistry

and

IUPAC–IUB Commission on Biochemical Nomenclature

1971

LONDON
BUTTERWORTHS

P.A.C.—31—1/2—M

DEFINITIVE RULES FOR NOMENCLATURE OF STEROIDS†

CONTENTS

INTRODUCTION

The rules for steroid nomenclature originate from a discussion held at the Ciba Foundation in London, England, in 1950 between the representatives of many schools. These were published in *Chem. & Ind., London*, (1951) pp SN 1–11, and also in French and German. They were subsequently taken over by the International Union of Pure and Applied Chemistry and published in an official form in the *Comptes Rendus* of the Zürich meeting in 1952

† These Rules shall be known as the IUPAC–IUB 1971 Definitive Rules for Steroid Nomenclature.

These Rules are issued by the IUPAC Commission on the Nomenclature of Organic Chemistry and by the IUPAC–IUB Commission on Biochemical Nomenclature.

Those who have served on the Commission on the Nomenclature of Organic Chemistry for varying periods during 1967–71 are the following. Present members are shown by an asterisk*. P. E. VERKADE (Chairman to 1971), N. LOZAC'H* (Chairman from 1971), K. BLÁHA*, L. C. CROSS*, G. M. DYSON, S. P. KLESNEY*, W. KLYNE*, K. L. LOENING*, H. S. NUTTING, J. RIGAUDY*, S. VEIBEL*. Associate members: R. S. CAHN, H. GRÜNEWALD*, K. HIRAYAMA*. Observer: K. A. JENSEN*.

Those who have served on the Commission on Biochemical Nomenclature for varying periods during 1967–71 are the following. Present members are shown by an asterisk*. O. HOFFMANN-OSTENHOF* (Chairman), A. E. BRAUNSTEIN*, W. E. COHN*, J. S. FRUTON, B. HORECKER*, P. KARLSON*, B. KEIL*, W. KLYNE*, C. LIÉBECQ*, E. C. SLATER, E. C. WEBB*, W. J. WHELAN*. Observer: S. VEIBEL*.

Comments on and suggestions for future revisions of these Rules should be sent to: Professor N. LOZAC'H. Ecole nationale supérieure de Chimie, 5 Avenue d'Edimbourg, F-14 Caen, France, or Professor O. HOFFMANN-OSTENHOF, Lehrkanzel für Biochemie der Universität Wien, Währingerstrasse 38, 1090 Vienna, Austria, or to any present member of the Commissions named above.

[also *IUPAC* 1957 *Rules for Nomenclature of Steroids*, Butterworths: London (1958); 2nd ed. 1966, pp 71–82; and numerous reprints and translations, including *J. Am. Chem. Soc.* **82**, 5577 (1960)].

In 1960 a group of specialists under the chairmanship of Professor T. Reichstein, including representatives of the IUPAC Commissions on the Nomenclature of Organic Chemistry and of Biochemical Nomenclature, met in Basle, Switzerland, for discussions of amendments and additions to the Rules. Agreement was not reached on all the points discussed, and the results of this meeting were therefore published in discussion form in the *IUPAC Information Bulletin*, No. 11. They have generally been referred to as the 'Basle Proposals'.

Since then, many points in the Basle Proposals have become almost universally accepted in the literature. In 1965 the two International Commissions concerned, namely, the IUPAC Commission on the Nomenclature of Organic Chemistry and the Commission on Biochemical Nomenclature (now jointly responsible to IUPAC and IUB), decided that the time had come for as many as possible of the Basle Proposals to be formulated as rules. Accordingly Tentative Rules were formulated and published in *Biochim. Biophys. Acta*, **164**, 453–486 (1968), in *IUPAC Information Bulletin*, No. 33 (1968), and elsewhere. These Rules have subsequently been studied by the two Commissions and amended on a number of (mostly minor) points.

The Definitive Rules include: all the original Rules, mostly renumbered (with additions and amendments arising from the Basle Proposals or from current practice in the literature); and most of the Basle Proposals, namely, those that have been generally accepted. Further, adoption of the sequence-rule procedure* for general stereochemical descriptions in much of the chemical literature has permitted its introduction now also for some sections of steroid nomenclature that were previously in dispute or intractable.

GENERAL APPLICATION

Although these Rules are called 'Rules for Nomenclature of Steroids', many of the principles therein have become universally accepted also in diterpene and triterpene chemistry; also to some extent for sesquiterpenes and for several groups of alkaloids. It is suggested that the same principles may be applied to a number of other specialized groups of natural products, perhaps without the need for further official rules, so long as the basic ideas are followed. These principles include: (i) clear definition of stem names and the stereochemistry implied in them; (ii) systematic application of the rules of general organic chemical nomenclature, with modifications where special considerations make this necessary; (iii) application of the methods of skeletal modification given in these Rules, viz. the use of homo and nor for, respectively, stepwise expansion and contraction of ring systems; the use of seco for reductive fission of ring systems; and the use of *abeo* for formal bond

* R. S. Cahn, C. K. Ingold and V. Prelog, *Angew. Chem. Intern. Ed.* **5**, 385 (1966) (in English); *Angew. Chem.* **78**, 413 (1966) (in German); for a partial simplified account see R. S. Cahn, *J. Chem. Educ.* **41**, 116 (1964). See also 'IUPAC 1968 Tentative Rules for the Nomenclature of Organic Chemistry, Sections E, Fundamental Stereochemistry', *IUPAC Information Bulletin*, No. 35, 71–80 (1969).

migrations (this flexible concept was first proposed by Professor D. H. R. Barton at an informal meeting of terpene chemists convened by the Chemical Society in London, England).

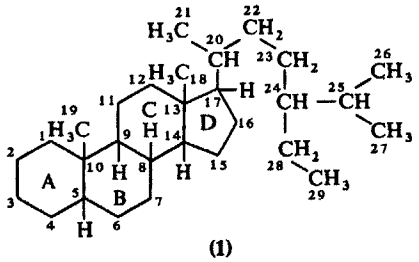
RULES

Rules are numbered **2S–1**, **2S–2**, **2S–3**, etc., the first '2' denoting that this is the second or revised set of rules. The numbers of the corresponding previous rules, where they exist, are included for comparison.

GENERAL

Rule 2S–1 (expanded from Rules S–1 and S–2)

1.1. Steroids are numbered and rings are lettered as in Formula (1). If one of the two methyl groups attached to C-25 is substituted it is assigned the lower

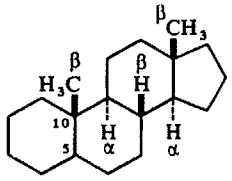


(1)

number (26); if both are substituted, that carrying the substituent cited first in the alphabetical order is assigned the lower number [cf. IUPAC Rule* **C–15.11(e)**]. For trimethyl steroids see Rule **2S–2.3**, Note c.

1.2. If one or more of the carbon atoms shown in (1) is not present and a steroid name is used, the numbering of the remainder is undisturbed.

1.3. For a steroid the name, including stereochemical affixes, and its structural formula (see Rule **2S–1.4**), denote the absolute configuration at each asymmetric centre (see also Rule **2S–1.5**). When the configuration at one or more centres is not known, this is indicated by Greek letter(s) ξ (xi) prefixed by the appropriate numeral(s).

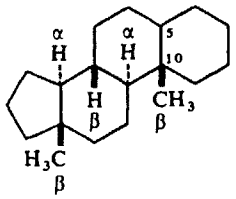


(2)

* *IUPAC Nomenclature of Organic Chemistry, Section A, B and C*, 1971, Butterworths: London.

1.4. When the rings of a steroid are denoted as projections on to the plane of the paper, the formula is normally to be oriented as in (2). An atom or group attached to a ring depicted as in the orientation (2) is termed α (alpha) if it lies below the plane of the paper or β (beta) if it lies above the plane of the paper. In formulae, bonds to atoms or groups lying below the plane of the paper are shown as broken (– – – –) lines, and bonds to atoms or groups lying above the plane of the paper are shown as solid lines preferably thickened (———). Bonds to atoms or groups whose configuration is not known are denoted by wavy lines (~~~~~).

Notes: (1) Projections of steroid formulae should not be oriented as in Formula (3), (4) or (5) unless circumstances make it obligatory.



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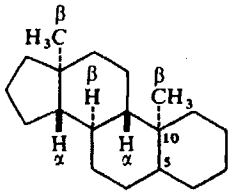

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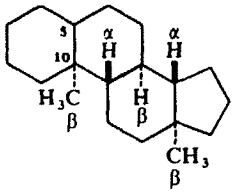
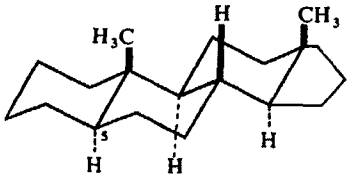


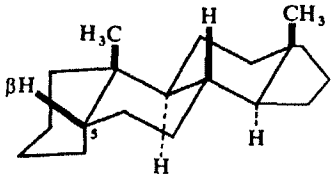
(5)

(2) With the preferred orientation (2), and with (3), α bonds appear as broken lines and β bonds as solid (thickened) lines. The reverse is true for (4) and (5). Wavy lines denote ξ bonds for all orientations of the formula.

(3) A perspective representation of the stereochemistry of Formula (2) as in (2a) or (2b) may also be used.



(2a)
A 5α-steroid



(2b)
A 5β-steroid

(For the significance of the prefixes 5α- and 5β-, see Rule **2S–1.5.**)

DIAGNOSTIC AND STATISTICAL MANUAL OF MENTAL DISORDERS

FOURTH EDITION

TEXT REVISION

DSM-IV-TR™

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Schizophrenia and Other Psychotic Disorders

The disorders in this section include Schizophrenia, Schizophreniform Disorder, Schizoaffective Disorder, Delusional Disorder, Brief Psychotic Disorder, Shared Psychotic Disorder, Psychotic Disorder Due to a General Medical Condition, Substance-Induced Psychotic Disorder, and Psychotic Disorder Not Otherwise Specified. These disorders have been grouped together to facilitate the differential diagnosis of disorders that include psychotic symptoms as a prominent aspect of their presentation. Other disorders that may present with psychotic symptoms as associated features are included elsewhere in the manual (e.g., Dementia of the Alzheimer's Type and Substance-Induced Delirium in the "Delirium, Dementia, and Amnestic and Other Cognitive Disorders" section; Major Depressive Disorder, With Psychotic Features, in the "Mood Disorders" section). Despite the fact that these disorders are grouped together in this chapter, it should be understood that psychotic symptoms are not necessarily considered to be core or fundamental features of these disorders, nor do the disorders in this section necessarily have a common etiology. In fact, a number of studies suggest closer etiological associations between Schizophrenia and other disorders that, by definition, do not present with psychotic symptoms (e.g., Schizotypal Personality Disorder).

The term *psychotic* has historically received a number of different definitions, none of which has achieved universal acceptance. The narrowest definition of *psychotic* is restricted to delusions or prominent hallucinations, with the hallucinations occurring in the absence of insight into their pathological nature. A slightly less restrictive definition would also include prominent hallucinations that the individual realizes are hallucinatory experiences. Broader still is a definition that also includes other positive symptoms of Schizophrenia (i.e., disorganized speech, grossly disorganized or catatonic behavior). Unlike these definitions based on symptoms, the definition used in earlier classifications (e.g., DSM-II and ICD-9) was probably far too inclusive and focused on the severity of functional impairment. In that context, a mental disorder was termed "psychotic" if it resulted in "impairment that grossly interferes with the capacity to meet ordinary demands of life." The term has also previously been defined as a "loss of ego boundaries" or a "gross impairment in reality testing."

In this manual, the term *psychotic* refers to the presence of certain symptoms. However, the specific constellation of symptoms to which the term refers varies to some extent across the diagnostic categories. In Schizophrenia, Schizophreniform Disorder, Schizoaffective Disorder, and Brief Psychotic Disorder, the term *psychotic* refers to delusions, any prominent hallucinations, disorganized speech, or disorganized or catatonic behavior. In Psychotic Disorder Due to a General Medical Condition and in

Diagnostic criteria for Substance-Induced Psychotic Disorder *(continued)*

Code [Specific Substance]–Induced Psychotic Disorder:

(291.5 Alcohol, With Delusions; 291.3 Alcohol, With Hallucinations; 292.11 Amphetamine [or Amphetamine-Like Substance], With Delusions; 292.12 Amphetamine [or Amphetamine-Like Substance], With Hallucinations; 292.11 Cannabis, With Delusions; 292.12 Cannabis, With Hallucinations; 292.11 Cocaine, With Delusions; 292.12 Cocaine, With Hallucinations; 292.11 Hallucinogen, With Delusions; 292.12 Hallucinogen, With Hallucinations; 292.11 Inhalant, With Delusions; 292.12 Inhalant, With Hallucinations; 292.11 Opioid, With Delusions; 292.12 Opioid, With Hallucinations; 292.11 Phencyclidine [or Phencyclidine-Like Substance], With Delusions; 292.12 Phencyclidine [or Phencyclidine-Like Substance], With Hallucinations; 292.11 Sedative, Hypnotic, or Anxiolytic, With Delusions; 292.12 Sedative, Hypnotic, or Anxiolytic, With Hallucinations; 292.11 Other [or Unknown] Substance, With Delusions; 292.12 Other [or Unknown] Substance, With Hallucinations)

Specify if (see table on p. 193 for applicability by substance):

With Onset During Intoxication: if criteria are met for Intoxication with the substance and the symptoms develop during the intoxication syndrome

With Onset During Withdrawal: if criteria are met for Withdrawal from the substance and the symptoms develop during, or shortly after, a withdrawal syndrome

298.9 Psychotic Disorder Not Otherwise Specified

This category includes psychotic symptomatology (i.e., delusions, hallucinations, disorganized speech, grossly disorganized or catatonic behavior) about which there is inadequate information to make a specific diagnosis or about which there is contradictory information, or disorders with psychotic symptoms that do not meet the criteria for any specific Psychotic Disorder.

Examples include

1. Postpartum psychosis that does not meet criteria for Mood Disorder With Psychotic Features, Brief Psychotic Disorder, Psychotic Disorder Due to a General Medical Condition, or Substance-Induced Psychotic Disorder
2. Psychotic symptoms that have lasted for less than 1 month but that have not yet remitted, so that the criteria for Brief Psychotic Disorder are not met
3. Persistent auditory hallucinations in the absence of any other features
4. Persistent nonbizarre delusions with periods of overlapping mood episodes that have been present for a substantial portion of the delusional disturbance
5. Situations in which the clinician has concluded that a Psychotic Disorder is present, but is unable to determine whether it is primary, due to a general medical condition, or substance induced

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IL-12, TNF-α, and Hormonal Changes during Late Pregnancy and Early Postpartum: Implications for Autoimmune Disease Activity during These Times

EX.D

ILIA J. ELENKOV*, RONALD L. WILDER*, VLADIMIR K. BAKALOV, AMREY A. LINK, MARIANA A. DIMITROV, SCOTT FISHER, MARIANNA CRANE, KEITH S. KANIK, AND GEORGE P. CHROUSOS

Arthritis and Rheumatism Branch (I.J.E., R.L.W., M.C., K.S.K.), National Institute of Arthritis and Musculoskeletal and Skin Diseases, Pediatric and Reproductive Endocrinology Branch (I.J.E., A.A.L., S.F., G.P.C.), National Institute of Child Health and Human Development, Developmental Endocrinology Branch (V.K.B.), National Institute of Child Health and Human Development, and Laboratory of Experimental and Computational Biology (M.D.), National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

Clinical observations indicate that some autoimmune diseases, such as rheumatoid arthritis and multiple sclerosis, frequently remit during pregnancy but exacerbate, or have their onset, in the postpartum period. The immune basis for these phenomena is poorly understood. Recently, excessive production of IL-12 and TNF-α was causally linked to rheumatoid arthritis and multiple sclerosis. We studied 18 women with normal pregnancies in their third trimester and during the early postpartum period. We report that during the third trimester pregnancy, *ex vivo* monocytic IL-12 production was about 3-fold and TNF-α production was approximately 40% lower than postpartum values. At the same time, urinary cortisol and norepinephrine excretion and serum levels of 1,25-dihydroxyvitamin were 2- to 3-fold higher than postpartum values. As shown previously, these hormones can directly suppress IL-12 and TNF-α production by monocytes/macrophages *in vitro*. We suggest that a cortisol-, norepinephrine-, and 1,25-dihydroxyvitamin-induced inhibition and subsequent rebound of IL-12 and TNF-α production may represent a major mechanism by which pregnancy and postpartum alter the course of or susceptibility to various autoimmune disorders. (*J Clin Endocrinol Metab* 86: 4933–4938, 2001)

IL-12, PRODUCED by antigen-presenting cells, is a major inducer of T helper 1 (Th1) responses by stimulating Th1 lymphocyte proliferation and differentiation and by inducing interferon (IFN)-γ production from natural killer and T cells (1, 2). Antigen-presenting cell-derived IL-12 and TNF-α, in concert with Th1 cell-derived IFN-γ, stimulate the activity of T cytotoxic and natural killer cells, and monocytes/macrophages, *i.e.* the major components of cellular immunity. IL-12 and TNF-α are considered major proinflammatory cytokines because they stimulate the synthesis of nitric oxide and other inflammatory mediators that drive chronic delayed-type inflammatory responses (2). On the other hand, the antiinflammatory cytokine IL-10 produced by monocytes/macrophages and Th2 cells promotes humoral immunity and inhibits monocyte/macrophage activation and the production of proinflammatory cytokines (1). Excessive production of IL-12 and TNF-α and a deficit of IL-10 appears to play a key role in the inflammatory activity and the tissue damage observed in organ-specific autoimmune diseases, such as rheumatoid arthritis (RA) and multiple sclerosis (MS) (3–5). Moreover, excessive IL-12 production is the pivotal factor in the proliferation and differentiation of pathogenic autoreactive Th1 effector cells in the experimental models of these diseases (6).

Some autoimmune diseases, such as RA and MS, often remit during pregnancy, particularly in the third trimester, but have an exacerbation or their onset during the postpartum period (7–10). The risk of developing new onset RA during pregnancy, compared with nonpregnancy, is decreased by about 70%. In contrast, the risk of developing RA is markedly increased in the postpartum period, particularly the first 3 months (odds ratio of 5.6 overall and 10.8 after first pregnancy) (10). Moreover, a substantial fraction (20–30%) of premenopausal onset RA develops within 1 yr of pregnancy (R. Wilder, unpublished observations). In women with multiple sclerosis, the rate of relapse declines during pregnancy, especially in the third trimester, increases during the first 3 months postpartum, and then returns to the prepregnancy rate (8). Although documented extensively, these observations remain poorly understood.

The third trimester of pregnancy and the early postpartum period are also known to be associated with abrupt changes of several hormones, including in tandem increases and decreases, respectively, of E2, progesterone, cortisol, and 1,25-dihydroxyvitamin D_3 (9, 11). Recently, we and others demonstrated that cortisol, catecholamines (norepinephrine and epinephrine), and 1,25-dihydroxyvitamin D_3 are potent inhibitors of IL-12 and TNF-α production by monocytes/macrophages *ex vivo* and *in vitro* (12–16). We hypothesized that during late pregnancy the increase of these hormones, and their rapid decline in the early postpartum period, may induce opposite changes in both IL-12 and TNF-α production (17). Therefore, we examined the production of IL-12 and TNF-α after lipopolysaccharide (LPS) stimulation of whole

Abbreviations: IFN, Interferon; LPS, lipopolysaccharide; MS, multiple sclerosis; NE, norepinephrine; RA, rheumatoid arthritis; Th, T helper.

blood cultures *ex vivo* and measured the levels of E2, progesterone, cortisol, 1,25-dihydroxyvitamin D_3, and catecholamines in women during gestation wk 33–36 and 3–6 wk after delivery.

Materials and Methods

Subjects

Eighteen healthy pregnant women between the ages of 20 and 40 yr and 18 age-matched, healthy, nonpregnant women participated in the study, which was approved by the institutional review board of the NIH. The pregnant women underwent testing during gestation wk 33–36 and 3–6 wk after delivery. They were enrolled in the study with the approval of their obstetricians. We screened each participant at the NIH Clinical Center by history, physical examination, and routine laboratory tests. All signed informed consents. The controls had their tests during the early and mid follicular phases of their menstrual cycle (d 3–8). All participants abstained from taking medications (except prenatal vitamins and iron supplements in pregnancy) during the week before the study. Blood specimens for hormone measurements were drawn after 1 h of rest between 1300 and 1400 h. Urine samples were collected for two 24-h periods during the preceding 2 d to measure free cortisol and catecholamine excretion rates.

Whole blood cultures

Ex vivo whole blood cytokine production assays were performed as described elsewhere (12). Blood was drawn into sodium-heparin-containing sterile tubes (Vacutainer, Becton Dickinson and Co., Lincoln Park, NJ) and processed within 45 min. The blood, diluted 1:5 with RPMI 1640 (supplemented with 1% glutamine and 50 μg/ml gentamicin) with no added exogenous serum, was divided into aliquots (1.0 ml) in 24-well cell culture plates (Costar, Cambridge, MA). To induce cytokine production, bacterial LPS was added at 1 μg/ml final concentration, and the samples were incubated in 5% CO_2 at 37 C for 18 h. After incubation, the blood was centrifuged, and the supernatant plasma was separated and stored in polypropylene tubes at −70 C until assayed.

The whole blood *ex vivo* cytokine assay, which has recently found favor elsewhere (18), has several advantages. This method avoids the isolation of leukocytes from whole blood that may cause activation and artifactual differences not present *in vivo*. The method also preserves the "natural environment" (including hormones) of cytokine-producing cells. Importantly, in comparison to methods using isolated peripheral blood mononuclear cells, the whole blood assay also shows less intra-individual variation. Less than 15% intraindividual variation of whole blood cytokine production is reported when subjects are sampled over time (18, 19) (Elenkov, I. J., R. L. Wilder, and G. P. Chrousos, unpublished observations). This contrasts with the wide (but stable) range of IL-12, TNF-α, and IL-10 secretion levels seen across healthy individuals (interindividual variation) (18–21), demonstrating that this test forms a good basis for the study of genetically or hormonally defined variation.

Monocytes/macrophages are the main IL-12-, TNF-α-, and IL-10-producing cells in LPS-stimulated whole blood (22). In view of the observed changes of monocyte/macrophage numbers during pregnancy (see *Results*), the whole blood cytokine production was corrected for monocyte/macrophage counts (pg per 10^6 monocytes/macrophages).

Cytokine assays

IL-12 p70, TNF-α, and IL-10 were measured using ELISA employing the multiple antibody sandwich principle (Quantikine, R&D Systems, Inc., Minneapolis, MN). IL-12 p70 ELISA recognizes specifically the biologically active IL-12 heterodimer without cross-reactivity with the individual subunits of the dimer (p35 and p40). The detection limits of the IL-12 p70 and the high sensitivity IL-12 p70 ELISA were 7.5 and 0.5 pg/ml, respectively, whereas they were 15.0 and 2.0 pg/ml for the TNF-α and IL-10 ELISA. The quality control parameters of these ELISAs were as follows: intraassay coefficient of variation (CV), 1.1–1.5%; interassay CV, 3.3–7.1%. Plates were read by a microplate reader (model 550, Bio-Rad Laboratories, Inc., Richmond, CA), and absorbance was transformed to cytokine concentration (pg/ml) using a standard curve computed by Microplate Manager III (Macintosh Data Analysis Software, Bio-Rad Laboratories, Inc.).

Hormonal measurements

E2 was measured by RIA after extraction and LH20 column chromatography. Intraassay CV was 4.5%, and interassay CV was 11%. Normal values for the follicular phase are 0.38–367 nmol/l. Progesterone was measured by RIA after extraction with hexane. Interassay CV was 6.7%, and intraassay CV was 4.5%. Normal values for the follicular phase are 0.003–0.03 nmol/l. 1,25-Dihydroxyvitamin D_3 was measured by cartridge extraction and RRA. Intraassay and interassay CVs were 10%. Normal values are 53–161 pmol/l (Mayo Clinical Laboratories, Rochester, MN). Twenty-four-hour urinary excretion of free cortisol was measured after extraction by chemiluminescent competitive protein binding assay. Intraassay and interassay CVs were 4.4%. Normal values are 66–298 nmol/24 h (Mayo Clinic Laboratories). Twenty-four-hour urinary excretion of epinephrine and norepinephrine (NE) were measured by HPLC with electrochemical detection. Intraassay and interassay CVs were 3.5 and 4.0, respectively. Normal values are 0–109 and 89–473 nmol/24 h, respectively (Mayo Clinic Laboratories).

Data analysis

All data are presented as means ± SE. ANOVA was done with Statistica (version 5.5, StatSoft, Inc., Tulsa, OK). Pregnancy and postpartum values of the same individuals were compared by repeated measures ANOVA. Values from healthy age-matched control subjects were compared with those of pregnancy and postpartum subjects using one-way ANOVA.

Results

Blood count changes during pregnancy and postpartum

Pregnancy was associated with an increase of white blood cell counts compared with healthy, nonpregnant controls and the postpartum state ($8.8 \pm 0.5 \times 10^3$ mm^3 *vs.* $6.0 \pm 0.3 \times 10^3$ mm^3, both in controls and postpartum; $P < 0.001$). This was attributable to a significant increase of polymorphonuclear leukocytes and monocytes (mean, $6.6 \pm 0.5 \times 10^3/\mu$l and $0.62 \pm 0.04 \times 10^3/\mu$l, respectively) during pregnancy compared with healthy matched nonpregnant controls (mean, $3.3 \pm 0.3 \times 10^3/\mu$l and $0.38 \pm 0.03 \times 10^3/\mu$l, respectively; $P < 0.001$) and the postpartum state (mean, $3.4 \pm 0.2 \times 10^3/\mu$l and $0.4 \pm 0.02 \times 10^3/\mu$l, respectively; $P < 0.001$). Pregnancy was also associated with a moderate but significant decrease ($P < 0.05$) of lymphocytes and eosinophil counts (mean, $1.8 \pm 0.1 \times 10^3/\mu$l and $0.09 \pm 0.01 \times 10^3/\mu$l, respectively) compared with control age-matched nonpregnant women (mean, $2.2 \pm 0.1 \times 10^3/\mu$l and $0.16 \pm 0.03 \times 10^3/\mu$l, respectively).

Decrease of IL-12 and TNF-α production during pregnancy

During pregnancy, whole blood IL-12 production was decreased 2-fold compared with the postpartum period (61.0 ± 10.5 *vs.* 120.7 ± 31.8 pg/ml, respectively). When corrected for monocyte count, the decrease of IL-12 production was more pronounced (>3 fold; Table 1). The individual changes of IL-12 production corrected for monocyte count in 18 women during pregnancy and their follow-up in the postpartum period are shown in Fig. 1. During pregnancy, 15 of the women had lower IL-12 production than postpartum. Of interest, we found a large interindividual variation of the "effect of pregnancy" on IL-12 production, *i.e.* 5 individuals

TABLE 1. Summary of LPS-induced IL-12 and TNF-α production *ex vivo* and hormone levels in 18 subjects during the third trimester of pregnancy and 3–6 wk after delivery and in 18 healthy age-matched nonpregnant women

Cytokine or Hormone	Nonpregnant age-matched controls	Pregnancy	Postpartum	Controls *vs.* pregnancy	Pregnancy *vs.* postpartum	Controls *vs.* postpartum
IL-12	142.1 ± 60.4	106.8 ± 21.4	340.1 ± 88.5	NS	<0.01	NS
TNF-α	5803.2 ± 971.6	5648.8 ± 484.5	7829.9 ± 870.4	NS	0.07	NS
24-h urinary free cortisol	109.1 ± 12.2	380.1 ± 31.9	134.2 ± 16.9	<0.001	<0.001	NS
24-h NE	184.9 ± 10.7	285.2 ± 17.4	132.6 ± 10.2	<0.001	<0.001	<0.01
25-dihydroxy-vitamin D	57.4 ± 6.3	87.4 ± 5.8	78.4 ± 4.6	<0.001	NS	<0.05
1,25-dihydroxy-vitamin D	111.9 ± 7.3	260.0 ± 20.6	94.1 ± 5.9	<0.0001	<0.0001	NS
E2	246.6 ± 29.2	53816.1 ± 11547.6	231.5 ± 151.9	<0.001	<0.001	NS
Progesterone	1.6 ± 0.4	380.1 ± 48.4	1.1 ± 0.4	<0.001	<0.001	NS

Data are presented as means ± SE. Cytokine production is corrected for monocyte number (pg/10^6 monocytes). Hormone levels are expressed as nmol/L, except for 1,25-dihydroxy-vitamin D, which is expressed in pmol/L; UFC (urinary free-cortisol) and urinary NE are expressed as nmol/24 h.

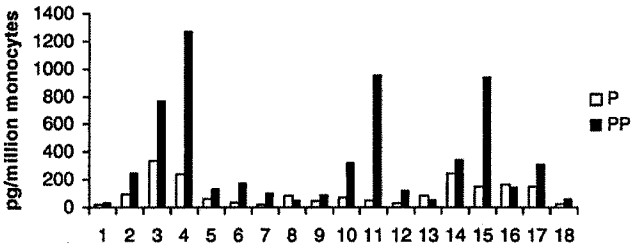

FIG. 1. Individual changes of IL-12 production by whole blood cultures stimulated with bacterial LPS *ex vivo* in 18 subjects during the third trimester of pregnancy and 3–6 wk after delivery. Cytokine production is corrected for monocyte number (pg/10^6 monocytes). P, Pregnancy; PP, postpartum.

had dramatic changes of IL-12 production, whereas others had moderate or minimal changes.

The production of TNF-α per monocyte was decreased by 40% during pregnancy compared with the postpartum period and narrowly failed to reach statistical significance ($P = 0.07$; Table 1). However, we observed similar levels of TNF-α production in pregnant *vs.* nonpregnant women (Table 1). There were no significant differences of LPS-induced IL-10 production by monocytes among the control, pregnant, and postpartum groups (data not shown).

Normal pregnancy is characterized by marked hormonal changes

The 24-h urinary cortisol excretion was increased about 4-fold during pregnancy compared with the nonpregnant state (Table 1), and in all cases it exceeded the upper limit of the reference values. After delivery, the cortisol excretion returned to normal levels. No changes in 24-h urinary excretion of epinephrine and dopamine were observed (data not shown), but we found that the 24-h urinary NE excretion was significantly increased during pregnancy and returned to baseline or lower levels in the postpartum period (Table 1).

As expected, the serum levels of E2 and progesterone were markedly increased during pregnancy. Postpartum, the ovarian hormone levels returned to normal follicular phase levels (Table 1). During pregnancy, plasma 25-hydroxyvitamin D_3 was increased by 50%, whereas 1,25-dihydroxyvitamin D_3 increased more than 2-fold (Table 1).

Cytokine production and hormone levels in a single individual before, during, and after pregnancy

We had the opportunity to follow the cytokine and hormonal changes of one woman (subject 4 in Fig. 1) as nonpregnant, during pregnancy, and postpartum (Fig. 2). She had a substantial decline in IL-12 production during pregnancy compared with the nonpregnant state. Three weeks after delivery, when cortisol, NE, E2, progesterone, and 1,25-dihydroxyvitamin D_3 returned to prepregnancy levels or lower, there was a notable rebound of LPS-induced IL-12 and TNF-α production.

E2 and progesterone do not affect IL-12, TNF-α, and IL-10 production

No data are available regarding whether E2 and progesterone are able to modulate the production of IL-12 by monocytes/macrophages. The significant changes of E2 and progesterone during pregnancy prompted us to study their direct effects in our assay system. Neither E2 nor progesterone at 10^{-5} to 10^{-11} M modulated the production of IL-12, TNF-α, or IL-10 in the LPS-stimulated human whole blood from five normal, nonpregnant individuals and three pregnant individuals (data not shown).

Discussion

We demonstrated that during late pregnancy, compared with the postpartum period, the capacity of monocytes to produce IL-12 was reduced more than 3-fold, whereas the capacity for TNF-α production was reduced by ~40%. The decreased production of these proinflammatory cytokines was paralleled by significant increases of cortisol, NE, 1,25-dihydroxyvitamin D_3, E2, and progesterone.

The pregnant women also had lower LPS-induced IL-12 production compared with age-matched controls, although the difference did not reach statistical significance (Table 1). The lack of significance in this case may reflect the large interindividual variability of monocytic IL-12 production across healthy individuals (Table 1). However, we observed a clear suppression of IL-12 production during pregnancy and a rebound in the postpartum when we followed a single individual through the nonpregnant, pregnant, and postpartum states (Fig. 2). Thus, because of the substantial interindividual variability of IL-12 production, larger and more

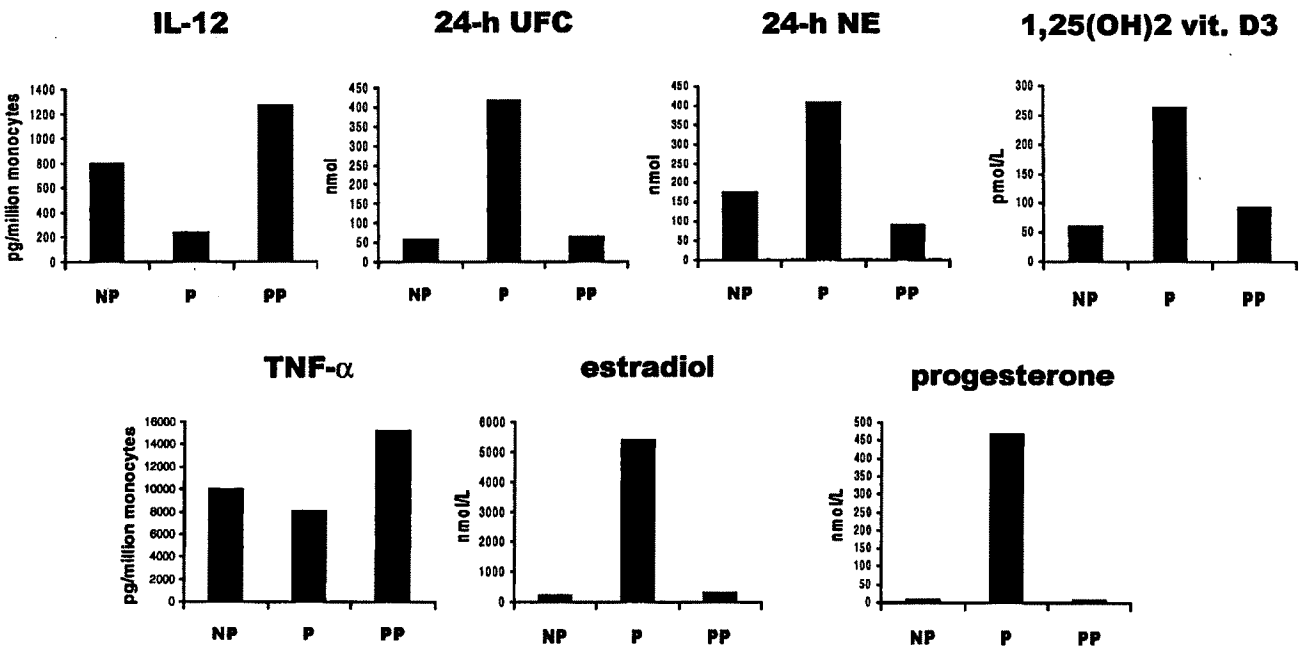

FIG. 2. Proinflammatory cytokine and hormonal changes in subject 4 (see Fig. 1) in the nonpregnant, pregnant, and postpartum states. Cytokine production is corrected for monocyte number (pg/10^6 monocytes), and hormone levels are expressed as nmol/l, except for 1,25-dihydroxyvitamin D_3, which is expressed as pmol/l. NP, Nonpregnant state; P, pregnancy; PP, postpartum; UFC, urinary free cortisol.

extended longitudinal studies are needed to address the differences between pregnancy and the nonpregnacy state.

Like others (23), we did not observe a difference of TNF-α production in pregnant *vs.* nonpregnant women. This might reflect the same factors described above for IL-12 production and an increase of soluble TNF receptors p55 and p75 in the third trimester of pregnancy, documented by Russell *et al.* (23). We did not find significant differences of LPS-induced IL-10 production by monocytes among the control, pregnant, and postpartum groups. Previous studies have shown increased production of IL-10 by peripheral blood mononuclear cells and placenta during pregnancy (24, 25). This discrepancy with our observations most likely reflects methodological differences. In the LPS-stimulated whole blood assay used by us, the primary source of IL-10 is the monocyte (22), whereas in the isolated peripheral blood mononuclear cell assay, which involves stimulation by mitogens, the primary source of IL-10 most likely is the lymphocyte.

The substantially increased urinary free cortisol excretion during the third trimester of pregnancy that returned to normal levels 3 wk after delivery indicates that late pregnancy is a state of adrenocortical activation, probably caused by the large amounts of CRH secreted by the placenta (11). We also found a significant increase of 24-h NE urinary excretion during pregnancy with a return to low normal levels in the postpartum period. This is consistent with observations in pregnant rats, in which a more than 2-fold increase of 24-h urinary excretion of NE has been described (26). Further studies are needed to elucidate whether these observations are linked to increased sympathetic nerve activity and/or reduced NE uptake during pregnancy, although, most likely, both take place (see also below).

The moderate increase of serum 25-hydroxyvitamin D_3 during pregnancy probably reflects the increased levels of serum vitamin D-binding proteins (27, 28). Like Seely *et al.* (28), we observed a more than 2-fold increase of the highly regulated serum 1,25-dihydroxyvitamin D_3 in the third trimester of pregnancy. These changes most likely result from increased conversion of 25-hydroxyvitamin D_3 to 1,25-dihydroxyvitamin D_3 in the human placenta, in addition to the increase of vitamin D-binding proteins (27, 28).

Recent evidence indicates that glucocorticoids, NE, and 1,25-dihydroxyvitamin D_3 potently inhibit the production of IL-12 and TNF-α by human monocytes/macrophages *in vitro* and *ex vivo* (12–16, 29). These hormones also inhibit the production of IL-2 and IFN-γ by Th1 cells (15, 29). In contrast, glucocorticoids do not affect the production of IL-10 by monocytes, but they potentiate IL-10 and IL-4 production by Th2 cells (12, 29, 30). Thus, the observed hormonal changes during pregnancy may explain the inhibition of monocytic IL-12 and TNF-α production. Furthermore, because IL-12 is extremely potent in enhancing IFN-γ and inhibiting IL-4 synthesis by T cells, the inhibition of IL-12 production may represent an important mechanism by which these hormones mediate a Th2 shift during pregnancy (Fig. 3).

We did not demonstrate a direct effect of E2 or progesterone on the production of IL-12, TNF-α, or IL-10 by human monocytes *ex vivo*. However, estrogens may affect cytokine production indirectly by enhancing the activity of the stress system, *i.e.* via increases in the secretion of cortisol and catecholamines (11). In addition, estrogens are potent inhibitors of the extraneuronal uptake of NE (uptake 2) (31), which may also explain the increased NE excretion in pregnancy demonstrated in our study. Therefore, estrogens may amplify the IL-12/TNF-α-inhibitory and Th2-facilitatory activities of cor-

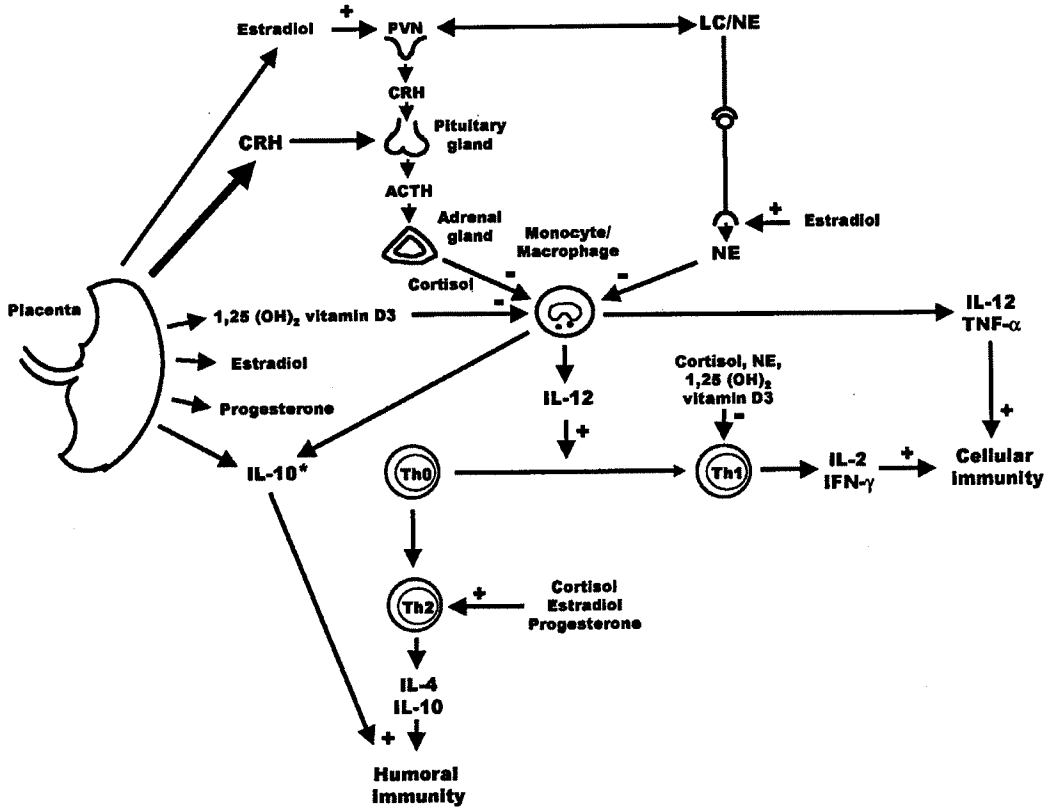

FIG. 3. A proposed simplified model of the role of different hormones in the regulation of innate and Th1 and Th2 cytokine profiles during pregnancy. Th1 cells primarily secrete IFN-γ and IL-2, which promote cellular immunity, whereas Th2 cells secrete primarily IL-4 and IL-10, which promote humoral immunity. During pregnancy, lymphocyte cytokine production is skewed toward the Th2 type: peripheral lymphocytes secrete less IFN-γ and IL-2 but more IL-4 and IL-10, particularly in the third trimester (24, 25). IL-12, a 75-kDa heterodimeric cytokine produced mostly by monocytes/macrophages, is a central inducer of Th1 responses and cell-mediated immunity by favoring Th1 cell proliferation and differentiation and by suppressing Th2 responses. Hypothalamic CRH stimulates the secretion of pituitary ACTH, which in turn triggers the secretion of cortisol from the adrenal cortex. During human pregnancy, the placenta is the major source of circulating CRH. The placenta also secretes IL-10 that may stimulate humoral and suppress cellular immunity. The sympathetic system innervates all peripheral tissues, including blood vessels and lymphoid organs. Upon activation, the sympathetic nerve terminals in these organs release NE locally and into the bloodstream. Cortisol, NE, 1,25-dihydroxyvitamin D_3, E2, and progesterone have multiple and divergent effects on the immune system. *Cortisol does not affect the production of IL-10 by monocytes/macrophages (see text). Note that *cortisol E2, and progesterone up-regulate IL-10 production by Th2 lymphocytes. In addition, E2 stimulates the activity of the CRH neurons and increases local NE concentrations by blocking its uptake. Thus, *in vivo*, E2 might amplify the effects of cortisol and NE. The net result of these complex hormonal effects is the suppression of IL-12 and TNF-α production, Th1 responses, and a Th2 shift. This hormonally induced Th2 shift may suppress Th1-related diseases such as RA and MS during pregnancy, whereas the rebound of IL-12 and TNF-α production and Th1 responses in the postpartum may facilitate the flares or the onset of these diseases. Note that several other factors, besides hormones (*e.g.* antibodies, soluble cytokine receptors, etc.), that most likely are also involved in the modulation of Th1/Th2 balance during pregnancy and postpartum, are not discussed here. LC, Locus ceruleus; PVN, paraventricular nucleus.

tisol and NE *in vivo* (Fig. 3). Furthermore, progesterone and estrogens up-regulate the production of IL-4 and IL-10 by Th2 cells *in vitro* (32, 33). Thus, an increase of estrogens and progesterone may also facilitate a Th2 shift during pregnancy by directly stimulating the production of IL-4 and IL-10 by Th2 cells (Fig. 3). This is consistent with recent data documenting increased IL-4 and IL-10 production by lymphocytes and the placenta during the third trimester of pregnancy (24, 25).

In conclusion, we demonstrated that human third trimester pregnancy, compared with the early postpartum period, is characterized by a reduction of the monocytic production of the Th1 type/proinflammatory cytokines IL-12 and TNF-α and by an increase of the secretion of cortisol, NE, and 1,25-dihydroxyvitamin D_3. Postpartum, when these hormones return to normal or low normal levels, the removal of their inhibitory effects may induce a rebound of IL-12 and TNF-α production and a Th1 shift.

The changes of Th1 type/proinflammatory cytokine production observed in this study may provide new understanding of the clinical observations that Th1-related diseases such as RA and MS frequently remit during pregnancy but exacerbate or have their onset in the postpartum period. Our study also suggests that some individuals have exaggerated postpartum Th1 type/proinflammatory cytokine rebound, raising the question of the factors that control this phenomenon. These individuals could be at greater than average risk for developing or exacerbating already existing

autoimmune diseases. Thus, further studies of the role of neuroendocrine factors in the regulation of IL-12, TNF-α/IL-10, and Th1/Th2 balance may suggest novel diagnostic and therapeutic approaches for these diseases.

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Address all correspondence and requests for reprints to: Dr. Ilia J. Elenkov, Division of Rheumatology, Immunology, and Allergy, Georgetown University Medical Center, 3800 Reservoir Road NW, Washington, D.C. 20007-2197. E-mail: ije@gunet.georgetown.edu.

* These authors contributed equally to this work.

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Review Article

Hormonal Changes in the Postpartum and Implications for Postpartum Depression

VICTORIA HENDRICK, M.D.
LORI L. ALTSHULER, M.D.
RITA SURI, M.D.

The months following childbirth are a time of heightened vulnerability to depressive mood changes. Because of the abrupt and dramatic changes occurring in hormone levels after delivery, many studies have examined the role of hormonal factors in postpartum depression. The authors review the literature on potential hormonal etiologies in postpartum depression, in particular for progesterone, estrogen, prolactin, cortisol, oxytocin, thyroid, and vasopressin. While evidence for an etiologic role is lacking for most hormones, changes in certain hormonal axes may contribute to depressive mood changes in some women following childbirth. (Psychosomatics 1998; 39:93–101)

The weeks following childbirth are a time of vulnerability to depressive symptomatology in women.[1,2] The literature on postpartum depression has inconsistently defined its time of onset from between 4 weeks and 6 months following delivery. DSM-IV, in an attempt to define the syndrome more rigorously, applies the term "postpartum onset" to depression occurring within 4 weeks of delivery. Most epidemiologic studies have not used this strict criterion. When defined as depression occurring in the first 6 months after delivery, rates are as high as 22%,[3] but drop to 12% to 16% if defined more narrowly as occurring in the first 6 to 9 weeks postpartum.[3,4]

Aside from the postpartum specifier, DSM-IV's criteria for postpartum depression are no different from those of a major depressive episode. However, in comparison with depression occurring at other times in women's lives, guilt and agitation appear to occur more frequently in cases of postpartum depression, and suicidality is less common.[5]

Risk factors for postpartum depression include a family history and a personal history of major depression[6] and depressive symptomatology during pregnancy.[7] Marital discord and stressful child care events (e.g., health problems in the baby) also increase the likelihood of postpartum depression.[6,7] A number of studies have explored whether specific biological characteristics may underlie depression in the postpartum, but with equivocal results. This article reviews the literature on hormonal factors that have been postulated as etiologic in postpartum depression.

HORMONAL EVENTS IN PREGNANCY AND POSTPARTUM

During pregnancy, levels of estrogens (estradiol, estriol, and estrone) and progesterone rise stead-

Received January 24, 1997; revised May 25, 1997; accepted June 6, 1997. From the University of California, Los Angeles (UCLA), Neuropsychiatric Institute and Hospital, and West Los Angeles Veterans Affairs Medical Center, Los Angeles, California. Address reprint requests to Dr. Hendrick, UCLA Neuropsychiatric Institute and Hospital, 300 Medical Plaza, Suite 1544, Los Angeles, CA 90095–6968.

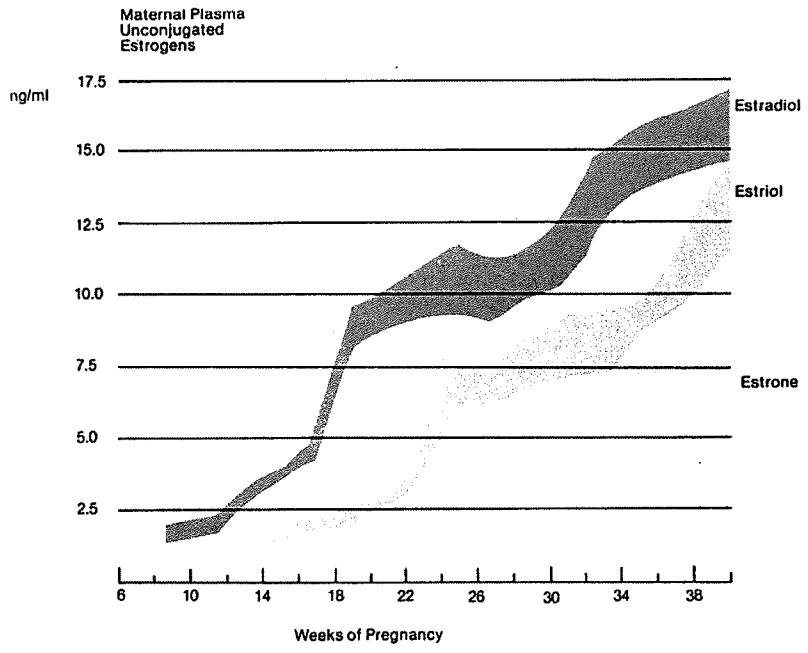
ily (see Figure 1 and Figure 2),[8] in large part as a result of placental production of these hormones. With removal of the placenta at delivery, estrogen and progesterone levels drop sharply, reaching pregravid levels by the fifth postpartum day. Levels of beta-endorphin, human chorionic gonadotrophin, and cortisol also rise across pregnancy, reaching a maximum near term and declining at delivery.

High estrogen levels during pregnancy stimulate production of thyroid hormone-binding globulin, leading to a rise in levels of bound T_3 (triiodothyronine) and T_4 (thyroxine) and a simultaneous drop in levels of free T_3 and T_4. In consequence, thyroid-stimulating hormone (TSH) increases to compensate for the low free-thyroid hormones, and free T_3 and T_4 thus remain within the normal range.[9] With the drop in thyroid-binding globulin following delivery, levels of total T_3 and T_4 drop, whereas free T_3 and T_4 remain relatively constant. Prolactin levels rise during pregnancy, peak at delivery and, in nonlactating women, return to pregravid levels within 3 weeks postpartum. By inducing the release of oxytocin, a hormone that stimulates pituitary lactotrophic cells, breast-feeding maintains high prolactin levels. Even in breast-feeding women, however, prolactin levels eventually return to pregravid levels.

GONADAL STEROIDS

Estradiol and estriol are biologically active forms of estrogen that are produced by the placenta and rise during pregnancy by 100-fold and 1,000-fold, respectively. Because synthesis of estriol results from metabolic activity of the fetal liver, it is produced in high concentrations during pregnancy. Animal studies have demonstrated that estradiol enhances neurotransmitter

FIGURE 1. Rise in levels of estrogens during pregnancy

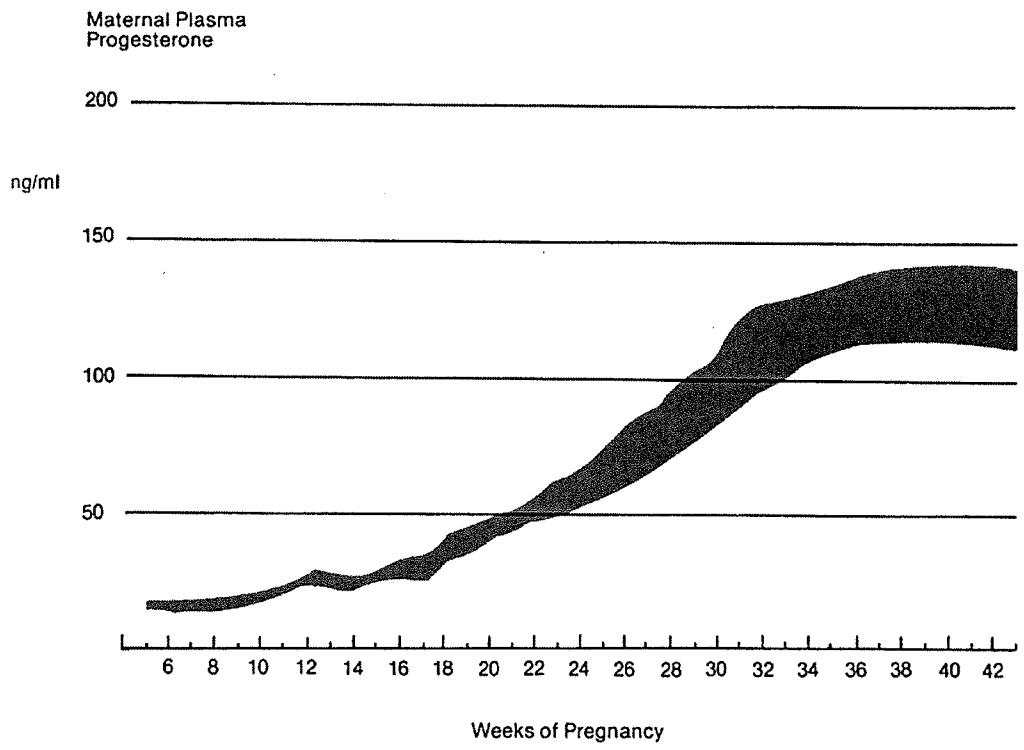


Source: Speroff L et al: Clinical Gynecologic Endocrinology and Infertility, 4th Edition. Baltimore, MD, Williams & Wilkins, 1983. Reprinted with permission.

function through increased synthesis and reduced breakdown of serotonin.[10] The abrupt decrease in estradiol levels following delivery may thus theoretically contribute to postpartum depression. However, a study of 182 childbearing women found no significant difference in the magnitude of change of total estradiol or of free estriol from late pregnancy to the puerperium in depressed and nondepressed women.[11] Total estradiol levels, measured on 9 separate days from Week 34 of gestation to Postpartum Day 8, were no different among the 2 groups of women, with the exception of a single significantly lower level of total estradiol at Week 36 in the women who developed postpartum depression. This finding is of unclear significance, particularly as the lower level was found in an antepartum rather than a postpartum sample. Other studies of total estradiol levels, obtained at various times between the first day and the eighth week following delivery, have found no difference in women with and without postpartum depression.[12,13] Levels of unbound (free) estradiol have not been studied in women with postpartum depression but merit examination, as the unbound form is biologically active.

Two recent studies have reported that estrogen supplementation significantly reduced postpartum depressive symptoms. The first was a small open study that included four women with a history of postpartum depression.[14] In the month following delivery the women received up to 10 mg of Premarin (estrogen-replacement therapy) daily, equivalent to about 15 times the

FIGURE 2. Rise in level of progesterone during pregnancy



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usual dose for estrogen-deficiency symptoms, and thus required heparin (5,000 units bid) to prevent thromboembolic phenomena. Over a 12-month follow-up period, none of these women experienced a recurrence of postpartum depression, despite the expected risk of relapse of 35% to 60%. The small sample size (4 cases of postpartum depression) was a major limitation of the study. In the second, a double-blind placebo-controlled study of 61 women with major depression that developed within 3 months of delivery,[15] 80% of the patients receiving an estrogen patch had Edinburgh Postnatal Depression Scale scores under the threshold for major depression after 3 months of treatment, compared with 31% of the placebo-treated group. However, nearly half of the estrogen-treated patients were also on antidepressant medications, confounding the study results.

The sharp decline in progesterone levels following childbirth has also been implicated in postpartum mood changes, but the data are conflicting. A study of 27 women followed every 3 days for the first 6 weeks after delivery found a weak association between postpartum depression and the magnitude of change of progesterone.[12] Further studies, however, have failed to confirm a relationship between postpartum depression and blood levels of either total[16,17] or free progesterone.[11,18]

Salivary levels of progesterone have been examined on the premise that they reflect the free, biologically active, fraction of plasma progesterone concentrations. A study of 147 mothers at 6 to 8 weeks postpartum found that the depressed breast-feeding women had lower levels of salivary progesterone than the euthymic breast-feeding women.[13] Levels of salivary progesterone were higher, on the other hand, in depressed postpartum women who were bottle feeding. However, nursing may have influenced progesterone levels by suppressing menstrual cycling, confounding the results of the study. A prospective study of 120 women found no association between the levels or the magnitude of change of salivary progesterone and depression at Day 35 postpartum.[19] One report describes prophylactic efficacy of progesterone given postnatally, but this study lacked a control group.[20] No controlled studies exist to date of progesterone in the prophylaxis or treatment of postpartum depression.

THYROID HORMONES

The incidence of abnormal thyroid function rises slightly after childbirth. In the 6 months following delivery, women experience thyroid dysfunction at a rate of up to 7%,[21–23] compared with a rate of 3% to 4% in the general population.[24] Although thyroid dysfunction has not been identified in most women with postpartum depression,[25] it may play a role for a subgroup of women.[26–31] In a prospective study of 303 pregnant euthyroid women, 21 women (7%) developed postpartum thyroid disorders.[31] Depression was identified in 38% of these 21 mothers and resolved with treatment of the thyroid dysfunction.[31] Thus, in women with symptoms suggesting hypothyroidism (weight gain, cold intolerance, lethargy), measurement of thyroid function is an important part of the evaluation of postpartum depression.

Some postpartum women without overt thyroid dysfunction may nevertheless have thyroid pathology. Thyroid antibodies have been found in up to 11.6% of postpartum women.[27] The immunosuppressant effect of high cortisol levels during pregnancy may be followed by a "rebound" immune phenomenon after delivery, producing a high incidence of postpartum thyroid antibodies.[9] A double-blind study of 145 antibody-positive women and 229 antibody-negative women found a relationship between depression and postpartum antibody status.[27] At 6 weeks following delivery, 43% of the antibody-positive women had mild-to-moderate depressive symptoms, compared with 28% of the antibody-negative women. Depression was defined by a score of 17 or higher on the Hamilton Depression scale, a score of 13 or more on the Edinburgh postnatal depression scale, and a score of 11 or more on a hospital anxiety and depression scale. Antibody-positive women should be followed with thyroid function testing beyond the postpartum period, as many patients

with antithyroid antibodies go on to develop overt hypothyroidism within 4 years.[32] At this time, however, there does not appear to be a role for thyroid antibody testing in the postpartum, as the relationship between antibodies and depression is weak.

Diminished thyroid function may affect postpartum mood through its association with diminished central 5-HT (5-hydroxytryptamine [serotonin]) activity. Blood levels of 5-HT have been positively correlated with thyroid hormone levels,[33] and the prolactin and cortisol responses to the 5-HT agonist fenfluramine are blunted in hypothyroid patients compared with euthyroid controls, suggesting reduced central 5-HT activity.[34]

PITUITARY HORMONES

Prolactin rises from pregravid levels of 5–25 ng/ml to 140 ng/ml in late pregnancy and drops in the 3 weeks after delivery in nonlactating women. In breast-feeding mothers, prolactin levels remain high for several months but eventually decline to prepregnancy levels. Prolactin's role in psychopathology has been suggested by the association of anxiety, depression, and hostility in nonpregnant women with pathologic hyperprolactinemia compared with control subjects.[35] One study of 147 women at 6–8 weeks postpartum found lower prolactin levels in the depressed breast-feeding women than in the nondepressed breast-feeding women.[13] However, all levels remained within normal physiological ranges. The study did not control for the relationship between breast-feeding and sampling time. As prolactin levels increase following breast-feeding and nipple stimulation, this is a significant confound. A large prospective study that did control for breast-feeding, in addition to demographic and psychosocial variables, failed to find a relationship between prolactin levels and postpartum mood.[11]

Oxytocin and vasopressin, two posterior pituitary hormones that undergo changes in levels in the postpartum, have not been assessed for their relationship to postpartum depression. Oxytocin, which rises sharply at delivery and with breast-feeding, stimulates uterine muscle contraction at labor and promotes release of breast milk. In animal studies, oxytocin also appears to stimulate maternal behavior.

Vasopressin regulates blood pressure and electrolyte balance and has been found lower in urine, but not plasma, of postpartum women compared with the nonpuerperal women in a study that did not assess mood state.[36] While negative results have been found in studies of vasopressin levels in women with postpartum blues,[36,37] no studies have assessed its levels in postpartum depression.

CORTISOL

Cortisol levels peak in late pregnancy as a result of placental production of corticotropin releasing hormone, and fall abruptly at delivery. A number of studies have failed to find an association between plasma cortisol,[11,13,38,39] or urinary-free cortisol[11] and postpartum depression. One study that did note a positive association between morning serum cortisol levels at 6 weeks postpartum and degree of dysphoria in 26 women[29] was confounded by a lack of control for stressful life events and for timing of breast-feeding, factors that may produce an elevation or a reduction, respectively, of cortisol levels.[40]

A prospective study of 182 women followed from the second trimester of pregnancy until Postpartum Week 9 controlled for lactation and for demographic, psychiatric, social, life stress, and other variables. No association was observed between total cortisol, urinary-free cortisol, or dexamethasone-suppression test results and postpartum mood.[11] Thus, current data do not support an etiologic role for cortisol in the onset of postpartum depression. A prospective study of 17 healty euthymic women evaluated in the second trimester of pregnancy and followed to the 12th postpartum week similarly found no relationship between mood and cortisol levels but did observe a significantly greater and longer lasting blunting of adrenocorticotropic hormone (ACTH) response to corticotropin-releasing hormone in women who developed postpartum blues or postpartum depression

compared with women who remained euthymic.[41] The authors speculated that the hypercortisolism that characterizes late pregnancy (resulting from placental production of corticotropin-releasing hormone) produces adrenal suppression following delivery that, when sustained and severe, may contribute to depressive mood changes after delivery. This intriguing but small study, consisting of only one case of postpartum depression and seven cases of postpartum blues, merits examination with a larger sample size.

DISCUSSION AND CONCLUSIONS

The dramatic physiological events occurring after delivery have led researchers to speculate that postpartum mood disorders result from a biochemical or hormonal etiology. While certain hormones, such as estradiol and ACTH, merit further exploration, studies have been negative or contradictory for most biological variables thought to be etiologic. Thus, the literature to date does not consistently support any single biological etiology for postpartum depression.

Methodological problems in many studies may have led to the conflicting results. For example, blood sampling in many studies did not control for breast-feeding. Lactation not only influences levels of prolactin, progesterone, estrogen, oxytocin, and cortisol but also has been associated with changes in mood state, both positive[42] and negative.[43] Other variables seldom controlled for in the studies were the time of day when assays were obtained, seasonal variations in hormone levels, extent of sleep deprivation in the mother, and potential medication effects on hormone levels. Many studies assessed total hormone concentration rather than free, biologically active hormone levels. While the majority of studies measured the absolute levels of a biological factor, it may be the degree of change—in particular, the degree of change of free hormone—from pregnancy to the early postpartum that affects psychopathology. Changes in mood may also occur from extreme sensitivity to normal levels of hormones.

A limitation of studies assessing serum levels of hormones and other biological factors is that peripheral levels do not necessarily reflect central activity. For beta-endorphins, for example, the relationship between peripheral and cerebrospinal fluid concentrations is small.[44] Thyroid hormone measures similarly show little parallel with peripheral indices.[45,46] Thus, measurement techniques that reliably reflect central neurotransmission are necessary to better establish the relationship between postpartum mood changes and neurotransmitter activity. Central levels of steroid hormones, however, are reported to correlate with plasma levels.[47]

It is possible that no biological etiologies are specific to the postpartum, but rather the birth of a child may represent a major stressful life event that, in vulnerable women, precipitates a depressive episode. Clearly, psychosocial stressors contribute to the syndrome in many women: a lack of support, marital conflict, unemployment, an unplanned pregnancy, single motherhood, and younger age are some factors associated with postpartum depression.[2,42,48] Infant factors, including high levels of irritability and poor motor behavior, also increase the likelihood of maternal depression.[49,50] Future research on the biological factors that may underlie postpartum mood disorders should attempt to control for these variables, as they otherwise are likely to confound the data. Measures such as the Neonatal Behavioral Assessment Scale,[51] the Life Events and Difficulties Schedule,[52] and the Perceived Stress Scale[53] can be used for this purpose. Further variables that should be taken into account include personality traits in the mother, length of time and severity of the mother's depression, and qualititative aspects of the depression, including presence of obsessional or anxious features. These are factors that have been shown to predict likelihood of antidepressant response in nonpuerperal major depression but their role in influencing treatment outcome of postpartum depression has not been assessed. The genetic vulnerability that may underlie the development of depression in the postpartum is also worth investigating, for example, through the use of family studies of women with postpartum depression.

A significant problem in research on the etiology of postpartum depression is the heterogeneity of the syndrome. Depression arising 1 week postpartum may be etiologically different from depression developing 3 months after delivery or from depression that had its onset during the pregnancy but continued through the postpartum period. Further, a postpartum depression with anxious and obsessional features may be etiologically different from an anergic postpartum depression. Some authors have postulated that postpartum depressions exist in two distinct categories: cases in which the index episode occurs in the postpartum, and cases in which the postpartum depression represents a recurrence of a previous nonpuerperal depression.[54] Compared with the former, the latter group appears to have a greater likelihood of nonpuerperal recurrence[54,55] and thus may require closer long-term follow-up. Other treatment implications, such as differences in treatment outcome between the two groups, are not clear. To our knowledge, no studies have examined biological variables that may distinguish these two potentially distinct populations of postpartum depressed women.

Postpartum depression can produce significant distress to the new mother and her family and may have an adverse impact on the cognitive and emotional development of the child.[56,57] Further, a postpartum depression predisposes a woman to future psychopathology, particularly following subsequent deliveries. The identification of etiologic factors is therefore of great importance, to allow for better understanding of preventive and treatment strategies. With the increasing tendency for researchers to employ standardized rating instruments (Edinburgh Postnatal Depression Scale, etc.) and to adhere to stringent criteria for postpartum depression, research in the etiology of postpartum depression is likely to advance in coming years.

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